

In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 3, lines 1-5, line and replace it with the following paragraph:

The inventors have now identified the family of enzymes which reduce Cys_P-SO₂H Prxs. It involves a protein that comprises at least one catalytic site having the following motif: FXGCHR, with X = G or S (SEQ ID NO: 15), and which has a molecular weight of approximately 8 to 14 kDa.

Please delete the paragraph on page 3, line 38, to page 4, line 5, and replace it with the following paragraph:

Consequently, a subject of the present invention is the use of a protein called sulfiredoxin (Srx), which comprises at least one catalytic site having the following motif: FXGCHR, with X = G or S (SEQ ID NO: 15), for catalyzing the reduction of peroxyredoxins (Prxs) in their superoxide form Prx-Cys_P-SO₂H (peroxyredoxin cysteine sulfinic acid) to a thiol derivative (SH).

Please delete the paragraph on page 4, lines 12-23, and replace it with the following paragraph:

According to an advantageous embodiment of said use, said sulfiredoxin is a sulfiredoxin of a microorganism, a plant or a higher organism, which generally comprises between 80 and 170 amino acids and at least the catalytic site having the following motif: FXGCHR, with X = G or S (SEQ ID NO: 15). They have the following percentage identities and similarities with respect to one another:

- yeast/human: 32% identity and 67% similarity
- yeast/plants: 23% identity and 39% similarity

- yeast/mouse: 31% identity and 51% similarity
- yeast/fungi: 80% identity and 90% similarity.

Please delete the paragraph on page 6, lines 1-5, and replace it with the following paragraph:

A subject of the present invention is also an isolated peptide corresponding to the catalytic site of Srx, characterized in that it is defined by the following sequence: FXGCHR, with X = S (SEQ ID NO: 16).

Please delete the paragraph on page 6, lines 7-12, and replace it with the following paragraph:

A subject of the present invention is also anti-Srx antibodies, characterized in that they are obtained by suitable immunization of an animal with an Srx protein, defined by a sequence selected from the group consisting of SEQ ID NOS: 1-3, 5-6 and 8-10, or the peptide FXGCHR, with X = S (SEQ ID NO: 16).

Please delete the paragraph on page 10, lines 21-32, and replace it with the following paragraph:

A subject of the present invention is also a method of screening for diseases related to cancer, to ageing, to neurodegenerative diseases and to neuromuscular diseases, which method is characterized in that it comprises:

- immunodetection of the Srx protein in a biological sample to be tested, using an antibody obtained by suitable immunization of an animal with an Srx protein or the peptide FXGCHR, with X = G or S (SEQ ID NO: 15), after separation of total proteins by electrophoresis, then
- evaluation of the quality and of the amount of said Srx protein compared with a control Srx protein.

Please delete the paragraph on page 13, line 34, to page 14, line 2, and replace it with the following paragraph:

A subject of the present invention is also a method of reducing a product comprising at least two cysteines with redox activity, which method is characterized in that it comprises bringing said protein into contact with a sulfiredoxin (Srx), which comprises at least one catalytic site having the following motif: FXGCHR, with X = G or S (SEQ ID NO: 15), in the presence of ATP and of magnesium.

Please delete the paragraph on page 14, line 28, to page 15, line 3, and replace it with the following paragraph:

- figures 2 and 3 represent the comparison of the Srx1 sequences in various species; figure 2: *S. cerevisiae* (SEQ ID NO: 1), *C. albicans* (SEQ ID NO: 2), *S. pombe* (SEQ ID NO: 3), *H. sapiens* (SEQ ID NO: 4), *M. musculus* (SEQ ID NO: 5), *D. melanogaster* (SEQ ID NO: 6) and *A. thaliana* (SEQ ID NO: 7); the identical regions are boxed in; the catalytic site is located around the conserved cysteine, indicated by an asterisk; figure 3: *S. cerevisiae* (SEQ ID NO: 1), *H. sapiens* (SEQ ID NO: 4), *M. musculus* (SEQ ID NO: 5), *D. melanogaster* (SEQ ID NO: 6), *A. thaliana* (SEQ ID NO: 7), *T. elongatus* (SEQ ID NO: 8) and *Nostoc sp.* (SEQ ID NO: 9). The GenBank accession Nos. are indicated on this figure. The sequence alignment was carried out using the CLUSTALW program. The amino acids that are identical in approximately 65% of the sequences are boxed in. The Srx1 active site comprising a cysteine (black arrow) and the other cysteines (white arrow) are indicated;

Please delete the paragraph on page 16, lines 4-19, and replace it with the following paragraph:

- figure 7 illustrates the interaction between Tsa1 and Srx1 in a covalent (disulfide bridge) and noncovalent manner; figure 7a: Western blotting of the HA-tagged Srx1 protein (lanes 1, 2 and 3) or of HA-tagged Srx1^{C84S} (lane 4) expressed in a wild-type strain (WT) (lanes 1, 2, 4) or in Δ t_{sa1} cells (lane 3) treated for 15 min with H₂O₂ (500 μ M), after SDS-

PAGE electrophoresis carried out under reducing (R) (lane 2) or nonreducing (NR) (lanes 1, 3, 4) conditions; figure 7b: the proteins copurified with the Srx1 tagged with 6His (SEQ ID NO: 17) (lanes 2, 4) or the untagged Srx1 (lanes 1, 3) under nonreducing conditions are separated by SDS-PAGE under nonreducing (lanes 1, 2) or reducing (lanes 3, 4) conditions and visualized by Coomassie blue staining. The protein bands are identified by MALDI-TOF mass spectrometry as indicated;

Please delete the paragraph on page 16, lines 21-30, and replace it with the following paragraph:

- figure 8 shows that the Srx1 protein and ATP are required for the reduction of oxidized Tsa1 *in vitro* by Srx1; figures 8 a and b: Western blotting analysis of the reduced (SH) and superoxidized (SO₂H) forms of Myc-Tsa1 in Δ t_{SA1} cell lysates incubated for 15 min at 30°C with purified Srx1 and ATP, at the concentrations indicated; figure 8c: Western blotting analysis of the reduced (SH) and superoxidized (SO₂H) forms of 6His-Tsa1 (6xHis disclosed as SEQ ID NO: 17) incubated for 15 min at 30°C with purified Srx1, ATP (1 mM) and Mg⁺⁺ (1 mM), as indicated;

Please delete the paragraph on page 16, lines 32-34, and replace it with the following paragraph:

- figure 9 illustrates the role of hSrx1 in the reduction of 6His-Prx1 and 6His-Prx2 (6xHis disclosed as SEQ ID NO: 17) in their superoxidized forms.

Please delete the paragraph on page 17, line 28, to page 18, line 5, and replace it with the following paragraph:

The following fusion proteins:

- Srx1-HA: fusion protein comprising the fusion of two HA epitopes at the C-terminal of Srx1 and

- 6His-Srx1: protein from fusion between Srx1 and, at its N-terminal end, six histidine tags (**SEQ ID NO: 17**), are constructed by PCR in two steps: the nucleotide primers used for the PCR incorporate the sequence of one or other of the HA epitopes (defined by the commercial antibody recognizing the HA epitope 12CA5, Babco, MMS-101 R) and 6His (6 histidines) (**SEQ ID NO: 17**) and amplify the complete coding sequence of Srx1, flanked by 400 and 200 base pairs upstream and downstream of their sequence and cloned at the EcoRI site of the plasmid pRS316 (No. ATCC 77145) or of the plasmid pRS426 (No. ATCC 77107).

Please delete the paragraph on page 19, lines 13-23, and replace it with the following paragraph:

* For the in vitro reduction, either 3 μ l of lysate (2 mg/ml) of Δ srx1 cells treated with H₂O₂ comprising oxidized Myc-Tsa1, or oxidized and purified 6His-Tsa1 (**6xHis disclosed as SEQ ID NO: 17**) (0.5 mg), are added to the reaction buffer (RM) (final volume of 80 μ l) [Tris-Cl, pH 6.8 (50 mM), KCl (100 mM)] containing purified Srx1 expressed by a baculovirus, ATP and MgCl₂ at the concentrations indicated, and incubated for 15 minutes at 30°C. The 6His-Tsa1 (**6xHis disclosed as SEQ ID NO: 17**) is oxidized to cysteine-sulfinic acid by incubation in the RM buffer containing DTT (10 mM) and H₂O₂ (1 mM) for 30 min, and diluted 16 times the reaction medium.

Please delete the paragraph on page 19, line 34, to page 20, line 7, and replace it with the following paragraph:

6His-Tsa1 (**6xHis disclosed as SEQ ID NO: 17**) is expressed in *E. coli* BL21 cells from the plasmid pET28a-Tsa1 after induction with isopropylthio- β -D-galactopyranoside, in accordance with the manufacturer's recommendations (Stratagene). The cells are suspended in a lysis buffer [Tris-Cl, pH 6.8 (50 mM), KCl (100 mM), DTT (2 mM), imidazole (20 mM)], supplemented with phenylmethanesulfonyl fluoride (PMSF) (1 mM), and lysed by means of freezing-thawing cycles and sonication. The extracts are centrifuged for 30 min at 30 000 g and the supernatant is passed over a Ni-NTA agarose column (Qiagen). After

washing of the column with the lysis buffer, the Tsa1 is eluted with lysis buffer supplemented with imidazole (150 mM).

Please delete the paragraph on page 20, lines 15-29, and replace it with the following paragraph:

6His-Srx1 (**6xHis disclosed as SEQ ID NO: 17**) and Srx1 are expressed from the plasmid pRS426 in the Δ trr1 strain, devoid of the thioredoxin reductase gene which stabilizes disulfide bridges. The cells are cultured as far as the middle of the exponential phase ($OD_{600\ nm} = 0.8$) and treated with H_2O_2 (5 mM) for 5 min, washed twice in water supplemented with NEM (10 mM), frozen and lysed in an Eaton press in a buffer C [Tris-Cl, pH 8 (100 mM), NaCl (50 mM) EDTA-without protease inhibitor (Roche-Boehringer), PMSF (1 mM), imidazole (20 mM), NEM (10 mM)]. The cell extract is centrifuged for 1 h 30 min at 10 000 g and the supernatant is passed over a Ni-NTA column (Qiagen). After washing of the column with a buffer D [Tris-Cl, pH 8 (100 mM), NaCl (50 mM)] + imidazole (20 mM), the proteins are eluted with the buffer D + imidazole (30 mM).

Please delete the paragraph on page 23, line 38, to page 24, line 10, and replace it with the following paragraph:

(B) the Srx1 protein is purified under native conditions by means of a 6His tag (**SEQ ID NO: 17**), from Δ trr1 cells treated for 5 minutes with 5 mM of H_2O_2 ; the purified proteins are then separated on a reducing or nonreducing SDS-PAGE gel. The various proteins indicated were identified by mass spectrometry; the purified proteins separated under nonreducing and reducing conditions come from the Δ trr1 mutant strain containing a copy of the SRX1 gene (wells No. 1 and 3), and from the Δ trr1 mutant strain containing a tagged (HA) copy of the SRX1 gene (wells No. 2 and 4); the reference molecular weights (MW) are expressed in kDa.

Please delete the paragraph on page 29, lines 27-34, and replace it with the following paragraph:

The lysate of High Five cells overexpressing hSrx1 was used, *in vitro*, to test its activity for reducing the human peroxyredoxins Prx1 and Prx2 superoxidized in the sulfinic acid form (figure 9). 6HIS-Prx1 and 6HIS-Prx2 (**6xHis disclosed as SEQ ID NO: 17**) were expressed, purified and superoxidized according to the same method as Tsa1 in *S. cerevisiae*. The protocol and the method are identical to those of example 1 (points 1.3 and 1.4).

Please delete the paragraph on page 29, line 38, to page 30, line 5, and replace it with the following paragraph:

Figure 9 illustrates the results obtained and shows the ability of hSrx1, expressed from Baculovirus in High Five cells, to reduce the human peroxyredoxins 6His-Prx1 and 6His-Prx2 (**6xHis disclosed as SEQ ID NO: 17**) superoxidized in the cysteine sulfinic acid form. This reduction requires the presence of the cofactors ATP (1 mM) and Mg⁺⁺ (1 mM) and dithiothreitol (2 mM).